NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF CHLOROPRENE

(CAS NO. 126-99-8)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

September 1998

NTP TR 467

NIH Publication No. 98-3957

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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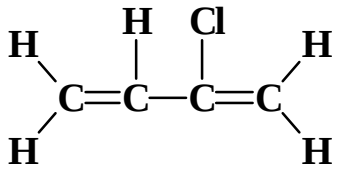
ABSTRACT



CHLOROPRENE

CAS No. 126-99-8

Chemical Formula: C_4H_5Cl Molecular Weight: 88.54

Synonyms: Chlorobutadiene; 2-chlorobuta-1,3-diene; 2-chloro-1,3-butadiene; β-chloroprene

Chloroprene is used almost exclusively in the manufacture of neoprene (polychloroprene). Chloroprene was chosen for study because it is a high-volume production chemical with limited information on its carcinogenic potential and because it is the 2-chloro analogue of 1,3-butadiene, a potent, multi-species, multi-organ carcinogen. Male and female F344/N rats and $B6C3F_1$ mice were exposed to chloroprene (greater than 96% pure) by inhalation for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Drosophila melanogaster*, and $B6C3F_1$ mice (bone marrow cells and peripheral blood erythrocytes).

16-DAY STUDY IN RATS

Groups of 10 male and 10 female F344/N rats were exposed to 0, 32, 80, 200, or 500 ppm chloroprene by inhalation, 6 hours per day, 5 days per week, for 16 days. Three 500 ppm males died on day 2 or 3 of the study. Mean body weight gains of 200 ppm males and females and 500 ppm females were significantly less than those of the chamber control groups. On the first day of exposure, rats exposed to 500 ppm were hypoactive and unsteady and had rapid shallow breathing. These effects were also observed to some degree in animals exposed to 200 ppm. After the second day of exposure, the effects in these groups worsened, and hemorrhage from the nose was observed.

A normocytic, normochromic, responsive anemia; thrombocytopenia; and increases in serum activities of alanine aminotransferase, glutamate dehydrogenase, and sorbitol dehydrogenase occurred on day 4 in 200 ppm females and 500 ppm males. Kidney weights of 80 and 500 ppm females were significantly greater than those of the chamber control group, as were the liver weights of 200 and 500 ppm females.

The incidences of minimal to mild olfactory epithelial degeneration of the nose in all exposed groups of males and females were significantly greater than those in the chamber control groups. The incidence of squamous metaplasia of the respiratory epithelium was significantly increased in 500 ppm males. The incidences of centrilobular to random hepatocellular necrosis in 500 ppm males and 200 ppm females were significantly greater than those in the chamber control groups.

16-DAY STUDY IN MICE

Groups of 10 male and 10 female B6C3F$_1$ mice were exposed to 0, 12, 32, 80, or 200 ppm chloroprene by inhalation, 6 hours per day, 5 days per week, for 16 days. All males and females exposed to 200 ppm died on day 2 or day 3 of the study. Mean body weight gains of males exposed to 32 or 80 ppm were significantly less than that of the chamber control group. Mice exposed to 200 ppm exhibited narcosis during exposure and were hypoactive with reduced body tone after the first day of exposure. In general, hematology and clinical chemistry parameters measured for exposed males and females were similar to those of the chamber control groups. Thymus weights of 80 ppm males and females were significantly less than those of the chamber control groups. Liver weights of 80 ppm females were significantly greater than those of the chamber control groups.

Increased incidences of multifocal random hepatocellular necrosis occurred in males and females exposed to 200 ppm. Hypertrophy of the myocardium, foci of hemorrhage, and mucosal erosion were observed in three males and three females exposed to 200 ppm. Squamous epithelial hyperplasia of the forestomach was observed in two males and two females exposed to 80 ppm. Thymic necrosis, characterized by karyorrhexis of thymic lymphocytes, was observed in all males and females in the 200 ppm groups.

13-WEEK STUDY IN RATS

Groups of 10 male and 10 female F344/N rats were exposed to chloroprene at concentrations of 0, 5, 12, 32, 80, or 200 ppm by inhalation, 6 hours per day, 5 days per week, for 13 weeks. One male exposed to 200 ppm died during the study. The final mean body weights and body weight gains of all exposed groups of males and females were similar to those of the chamber control groups. Clinical findings in 200 ppm males included red or clear discharge around the nose and eye region.

At week 13, a normocytic, normochromic, and nonresponsive anemia occurred in 200 ppm males and females. A thrombocytopenia occurred in 200 ppm males and females on day 2 and in 80 and 200 ppm females on day 22. However, at week 13, platelet counts rebounded and were minimally increased in 200 ppm males and females. On day 2, a minimal to mild increase in activated partial thromboplastin time and prothrombin time occurred in 200 ppm males and females. The 200 ppm males and females also had increased activities of serum alanine aminotransferase, glutamate dehydrogenase, and sorbitol dehydrogenase on day 22; these increases were transient, and by week 13 the serum activities of these enzymes were similar to those of the chamber controls. An alkaline phosphatase enzymeuria occurred in 200 ppm females on day 22; at week 13, an alkaline phosphatase enzymeuria occurred in 32, 80, and 200 ppm males and 200 ppm females. At week 13, a proteinuria occurred in 200 ppm males. Liver nonprotein sulfhydryl concentrations in male rats immediately following 1 day or 12 weeks of exposure to 200 ppm and in females exposed to 200 ppm for 12 weeks were significantly less than those of the chamber control groups.

Kidney weights of 200 ppm males and females and 80 ppm females were significantly greater than those of the chamber control groups. Sperm motility of 200 ppm males was significantly less than that of the controls. In neurobehavioral assessments, horizontal activity was increased in male rats exposed to 32 ppm or greater and total activity was increased in 32 and 200 ppm males.

Increased incidences of minimal to mild olfactory epithelial degeneration and respiratory metaplasia occurred in males and females exposed to 80 or 200 ppm. The incidence of olfactory epithelial degeneration in 32 ppm females was also significantly greater than that in the chamber control group. The incidence of hepatocellular necrosis in 200 ppm females was significantly greater than that in the chamber control group. Scattered chronic inflammation also occurred in the liver of male and female rats in the 200 ppm groups; the incidence in 200 ppm females was significantly greater than that in the chamber control group. The incidences of hemosiderin pigmentation were significantly increased in males and females exposed to 200 ppm.

13-WEEK STUDY IN MICE

Groups of 10 male and 10 female B6C3F$_1$ mice were exposed to chloroprene at concentrations of 0, 5, 12, 32, or 80 ppm by inhalation, 6 hours per day, 5 days per week, for 13 weeks. All male and female mice

survived to the end of the study. The final mean body weight and body weight gain of males exposed to 80 ppm were significantly less than those of the chamber control group.

Hematocrit concentrations of females exposed to 32 or 80 ppm and erythrocyte counts of 80 ppm females were significantly less than those of the chamber control group. Platelet counts of 32 and 80 ppm females were also greater than that of the chamber control group. Increased incidences of squamous epithelial hyperplasia of the forestomach occurred in males and females exposed to 80 ppm.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female F344/N rats were exposed to chloroprene at concentrations of 0, 12.8, 32, or 80 ppm by inhalation, 6 hours per day, 5 days per week, for 2 years.

Survival, Body Weights, and Clinical Findings

Survival of males exposed to 32 or 80 ppm was significantly less than that of the chamber control group. Mean body weights of males exposed to 80 ppm were less than those of the chamber controls after week 93. Masses of the torso were observed during the study in exposed female groups, and these clinical findings correlated with mammary gland fibroadenomas observed at necropsy.

Pathology Findings

The incidences of squamous cell papilloma and squamous cell papilloma or squamous cell carcinoma (combined) of the oral cavity in male rats exposed to 32 ppm and male and female rats exposed to 80 ppm were significantly greater than those in the chamber controls and exceeded the historical control ranges.

The incidences of thyroid gland follicular cell adenoma or carcinoma (combined) in males exposed to 32 or 80 ppm were significantly greater than that in the chamber control group and exceeded the historical control range. Although the incidences of follicular cell adenoma and follicular cell adenoma or carcinoma (combined) in 80 ppm females were not significantly greater than those in the chamber controls, they did exceed the historical control range for these neoplasms.

The incidences of alveolar epithelial hyperplasia of the lung were significantly greater in all exposed groups of males and females than in the chamber control groups. The incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in 80 ppm males were slightly greater than those in the chamber control group. Although these neoplasm incidences were not significant, they exceeded the historical control range. The incidence of alveolar/bronchiolar adenoma, although not significant, was greater in 80 ppm females than in the chamber control group.

The incidences of multiple fibroadenoma of the mammary gland in all exposed groups of females were greater than that in the chamber control group. The incidences of fibroadenoma (including multiple fibroadenoma) in 32 and 80 ppm females were significantly greater than that in the chamber controls. The incidences of fibroadenoma in the chamber control group and in all exposed groups of females exceeded the historical control range.

The severity of nephropathy in exposed groups of male and female rats was slightly greater than in the chamber controls. Renal tubule adenoma and hyperplasia were observed in males and females. Additional kidney sections from male and female control and exposed rats were examined to provide a clearer indication of the potential effects of chloroprene on the kidney. The combined single- and step-section incidences of renal tubule hyperplasia in 32 and 80 ppm males and 80 ppm females and the incidences of adenoma and adenoma or carcinoma (combined) in all exposed males were significantly greater than those in the chamber controls.

A slight increase in the incidence of transitional epithelium carcinoma of the urinary bladder was observed in 80 ppm females. In addition, one 32 ppm male had a transitional epithelium carcinoma and one 80 ppm male had a transitional cell papilloma. These findings are noteworthy because no urinary bladder neoplasms have been observed in chamber control male or female F344/N rats.

In the nose, the incidences of atrophy, basal cell hyperplasia, metaplasia, and necrosis of the olfactory epithelium in 32 and 80 ppm males and females and of

atrophy and necrosis in 12.8 ppm males were significantly greater than those in the chamber control groups. The incidences of chronic inflammation were significantly increased in males exposed to 12.8 or 32 ppm and in males and females exposed to 80 ppm. The incidences of fibrosis and adenomatous hyperplasia in 80 ppm males and females were significantly greater than those in the chamber controls. Generally, lesions in the nasal cavity were mild to moderate in severity.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female B6C3F$_1$ mice were exposed to chloroprene at concentrations of 0, 12.8, 32, or 80 ppm by inhalation, 6 hours per day, 5 days per week, for 2 years.

Survival, Body Weights, and Clinical Findings

Survival of males exposed to 32 or 80 ppm and of all exposed female groups was significantly less than that of the chamber controls. The mean body weights of 80 ppm females were significantly less than those of the chamber control group after week 75. Clinical findings included masses of the head, which correlated with harderian gland adenoma and/or carcinoma in 32 ppm males and 80 ppm males and females. Dorsal and lateral torso masses of female mice correlated with mammary gland neoplasms in 32 and 80 ppm females and subcutaneous sarcomas in 12.8, 32, and 80 ppm females.

Pathology Findings

The incidences of alveolar/bronchiolar neoplasms in the lungs of all groups of exposed males and females were significantly greater than those in the chamber control groups and generally exceeded the historical control ranges. The incidences of multiple alveolar/bronchiolar adenoma and alveolar/bronchiolar carcinoma were increased in all exposed groups of males and females. The incidences of bronchiolar hyperplasia in all exposed groups of males and females were significantly greater than those in the chamber control groups.

Male mice had a pattern of nonneoplastic liver lesions along with silver-staining helical organisms within the liver consistent with infection with *Helicobacter hepaticus*. An organism compatible with *H. hepaticus* was confirmed with a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)-based assay. In NTP studies with *H. hepaticus*-associated hepatitis, increased incidences of hemangiosarcoma have been seen in the livers of male mice. Therefore, hemangiosarcomas of the liver were excluded from the analyses of circulatory (endothelial) neoplasms in males in this study. Even with this exclusion, the combined occurrence of hemangioma or hemangiosarcoma at other sites was significantly increased at all chloroprene exposure concentrations in males and in 32 ppm females. Incidences of neoplasms at other sites in this study of chloroprene were not considered to have been significantly impacted by the infection with *H. hepaticus* or its associated hepatitis.

The incidences of harderian gland adenoma and harderian gland adenoma or carcinoma (combined) in males exposed to 32 or 80 ppm and females exposed to 80 ppm were significantly greater than those in the chamber controls. The incidences of harderian gland adenoma or carcinoma (combined) in 32 ppm males and 80 ppm males and females exceeded the historical control ranges.

The incidences of mammary gland carcinoma and adenoacanthoma or carcinoma (combined) in 80 ppm females were significantly greater than those in the chamber control group. The incidences of mammary gland carcinoma and of adenoacanthoma in 32 and 80 ppm females exceeded the historical control ranges. Multiple mammary gland carcinomas occurred in exposed females.

The incidences of hepatocellular carcinoma in all exposed female groups and hepatocellular adenoma or carcinoma (combined) in 32 and 80 ppm females were significantly greater than those in the chamber controls; in the 80 ppm group, the incidence exceeded the historical control ranges for carcinoma and adenoma or carcinoma (combined). The incidence of eosinophilic foci in 80 ppm females was also significantly greater than that in the chamber controls.

The incidences of sarcoma of the skin were significantly greater in all exposed groups of females than in the chamber controls. The incidences of sarcoma of the mesentery were also increased in all exposed groups of females.

The incidence of squamous cell papilloma in 80 ppm females was greater than that in the chamber controls; the difference was not significant, but the incidence exceeded the historical control range. Males also showed a positive trend in the incidence of squamous cell papilloma of the forestomach. In males and females exposed to 80 ppm, the incidences of hyperplasia of the forestomach epithelium were significantly greater than those in the chamber controls.

Carcinomas of the Zymbal's gland were seen in three 80 ppm females, and two carcinomas metastasized to the lung. Zymbal's gland carcinomas have not been reported in control female mice in the NTP historical database.

The incidence of renal tubule adenoma in 80 ppm males was greater than that in the chamber controls. Though this difference was not significant, the incidence of this rare neoplasm exceeded the historical control range. The incidences of renal tubule hyperplasia in males exposed to 32 or 80 ppm were significantly greater than that in the chamber controls. Additional sections of kidney were examined from control and exposed males to verify these findings. The combined single- and step-section incidence of renal tubule adenoma in 80 ppm males and the combined incidences of renal tubule hyperplasia in all groups of exposed male mice were greater than those in the chamber controls.

The incidences of olfactory epithelial atrophy, adenomatous hyperplasia, and metaplasia in 80 ppm males and females were significantly greater than those in the chamber controls. The incidences of hematopoietic proliferation of the spleen in 32 and 80 ppm males and in all groups of exposed females were significantly greater than those in the chamber controls.

GENETIC TOXICOLOGY

Chloroprene was not mutagenic in any of the tests performed by the NTP. No induction of mutations was noted in any of four strains of *S. typhimurium* in the presence or the absence of S9 metabolic activation enzymes, and no induction of sex-linked recessive lethal mutations was observed in germ cells of male *D. melanogaster* treated with chloroprene via feeding or injection. In male mice exposed to chloroprene by inhalation for 12 days over a 16-day period, no induction of chromosomal aberrations, sister chromatid exchanges, or micronucleated erythrocytes in bone marrow or peripheral blood occurred. Results of a second micronucleus assay in male and female mice after 13 weeks of exposure to chloroprene via inhalation were also negative.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of chloroprene in male F344/N rats based on increased incidences of neoplasms of the oral cavity; increased incidences of neoplasms of the thyroid gland, lung, and kidney were also attributed to chloroprene exposure. There was *clear evidence of carcinogenic activity* of chloroprene in female F344/N rats based on increased incidences of neoplasms of the oral cavity; increased incidences of neoplasms of the thyroid gland, mammary gland, and kidney were also attributed to exposure to chloroprene. Low incidences of urinary bladder neoplasms in male and female rats and lung neoplasms in female rats may also have been related to exposure to chloroprene.

There was *clear evidence of carcinogenic activity* of chloroprene in male B6C3F$_1$ mice based on increased incidences of neoplasms of the lung, circulatory system (hemangiomas and hemangiosarcomas), and harderian gland; increased incidences of neoplasms of the forestomach and kidney were also attributed to exposure to chloroprene. There was *clear evidence of carcinogenic activity* of chloroprene in female B6C3F$_1$ mice based on increased incidences of neoplasms of the lung, circulatory system (hemangiomas and hemangiosarcomas), harderian gland, mammary gland, liver, skin, and mesentery; increased incidences of neoplasms of the forestomach and Zymbal's gland were also attributed to exposure to chloroprene.

Exposure of male and female rats to chloroprene was associated with increased incidences of alveolar epithelial hyperplasia in the lung; nephropathy; and several nonneoplastic effects in the nose including olfactory epithelial atrophy, fibrosis, adenomatous hyperplasia, basal cell hyperplasia, chronic inflammation, respiratory metaplasia, and necrosis. Exposure of male and female mice to chloroprene was associated with increased incidences of bronchiolar hyperplasia and histiocytic cell infiltration in the lung; epithelial hyperplasia in the forestomach; renal

tubule hyperplasia (males only); several effects in the nose including olfactory epithelial atrophy, respiratory metaplasia, and adenomatous hyperplasia; and hematopoietic cell proliferation in the spleen.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 14. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 16.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Chloroprene

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Exposure concentrations	0, 12.8, 32, or 80 ppm by inhalation	0, 12.8, 32, or 80 ppm by inhalation	0, 12.8, 32, or 80 ppm by inhalation	0, 12.8, 32, or 80 ppm by inhalation
Body weights	80 ppm group less than chamber control group	Exposed groups similar to chamber control group	Exposed groups similar to chamber control group	80 ppm group less than chamber control group
Survival rates	13/50, 9/50, 5/50, 4/50	29/49, 28/50, 26/50, 21/50	27/50, 27/50, 14/50, 13/50	35/50, 16/50, 1/50, 3/50
Nonneoplastic effects	Lung: alveolar epthelial hyperplasia (5/50, 16/50, 14/49, 25/50) Kidney: severity of nephropathy (2.8, 3.0, 3.1, 3.5) Nose (olfactory epithelium): atrophy (3/50, 12/50, 46/49, 48/49); fibrosis (0/50, 0/50, 0/49, 47/49); adenomatous hyperplasia (2/50, 0/50, 1/49, 42/49); basal cell hyperplasia (0/50, 0/50, 38/49, 46/49); chronic inflammation (0/50, 5/50, 9/49, 49/49); metaplasia (6/50, 5/50, 45/49, 48/49); necrosis (0/50, 11/50, 26/49, 19/49)	Lung: alveolar epithelial hyperplasia (6/49, 22/50, 22/50, 34/50) Kidney: severity of nephropathy (1.9, 2.0, 2.0, 2.2) Nose (olfactory epithelium): atrophy (0/49, 1/50, 40/50, 50/50); fibrosis (0/49, 0/50, 0/50, 49/50); adenomatous hyperplasia (0/49, 0/50, 0/50, 27/50); basal cell hyperplasia (0/49, 0/50, 17/50, 49/50); chronic inflammation (0/49, 0/50, 2/50, 33/50); metaplasia (0/49, 1/50, 35/50, 50/50); necrosis (0/49, 0/50, 8/50, 12/50)	Lung: bronchiolar hyperplasia (0/50, 10/50, 18/50, 23/50); histiocytic cell infiltration (7/50, 8/50, 11/50, 22/50) Forestomach: epithelial hyperplasia (4/50, 6/48, 7/49, 29/50) Kidney: renal tubule hyperplasia (0/50, 4/49, 5/50, 5/50) Nose (olfactory epithelium): atrophy (7/50, 8/48, 7/50, 49/50); metaplasia (6/50, 5/48, 5/50, 49/50); adenomatous hyperplasia (3/50, 2/48, 2/50, 48/50) Spleen: hematopoietic cell proliferation (26/50, 22/49, 35/50, 31/50)	Lung: bronchiolar hyperplasia (0/50, 15/49, 12/50, 30/50); histiocytic cell infiltration (1/50, 14/49, 18/50, 23/50) Forestomach: epithelial hyperplasia (4/50, 3/49, 8/49, 27/50) Nose (olfactory epithelium): atrophy (6/50, 5/49, 4/49, 47/50); metaplasia (2/50, 3/49, 1/49, 44/50); adenomatous hyperplasia (2/50, 3/49, 0/49, 44/50) Spleen: hematopoietic cell proliferation (13/50, 25/49, 42/49, 39/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Chloroprene

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Neoplastic effects	Oral cavity: squamous cell papilloma (0/50, 2/50, 4/50, 10/50); squamous cell papilloma or squamous cell carcinoma (0/50, 2/50, 5/50, 12/50) Thyroid gland: follicular cell adenoma or carcinoma (0/50, 2/50, 4/49, 5/50) Lung: alveolar/ bronchiolar carcinoma (0/50, 2/50, 1/49, 4/50); alveolar/bronchiolar adenoma or carcinoma (2/50, 2/50, 4/49, 6/50) Kidney: renal tubule adenoma (extended evaluation - 1/50, 6/50, 6/50, 7/50; standard and extended evaluations combined - 1/50, 7/50, 6/50, 8/50); renal tubule adenoma or carcinoma (extended evaluation - 1/50, 7/50, 6/50, 7/50; standard and extended evaluations combined - 1/50, 8/50, 6/50, 8/50)	Oral cavity: squamous cell papilloma (1/49, 2/50, 2/50, 7/50); squamous cell papilloma or squamous cell carcinoma (1/49, 3/50, 5/50, 11/50) Thyroid gland: follicular cell adenoma or carcinoma (1/49, 1/50, 1/50, 5/50) Mammary gland: fibroadenoma (24/49, 32/50, 36/50, 36/50) Kidney: renal tubule adenoma or carcinoma (standard and extended evaluations combined - 0/49, 0/50, 0/50, 4/50)	Lung: alveolar/ bronchiolar adenoma (8/50, 18/50, 22/50, 28/50); alveolar/ bronchiolar carcinoma (6/50, 12/50, 23/50, 28/50); alveolar/ bronchiolar adenoma or carcinoma (13/50, 28/50, 36/50, 43/50) Circulatory system: hemangiosarcoma (3/50, 13/50, 22/50, 19/50); hemangiosarcoma (excludes liver) (1/50, 11/50, 16/50, 15/50); hemangioma or hemangiosarcoma (3/50, 14/50, 23/50, 21/50); hemangioma or hemangiosarcoma (excludes liver) (1/50, 12/50, 18/50, 17/50) Harderian gland: adenoma (2/50, 5/50, 8/50, 10/50); adenoma or carcinoma (2/50, 5/50, 10/50, 12/50) Forestomach: squamous cell papilloma (1/50, 0/50, 2/50, 4/50) Kidney: renal tubule adenoma (extended evaluation 0/50, 1/49, 2/50, 6/50); standard and extended evaluations combined - 0/50, 2/49, 3/50, 9/50)	Lung: alveolar/ bronchiolar adenoma (2/50, 16/49, 29/50, 26/50) alveolar/ bronchiolar carcinoma (2/50, 14/49, 16/50, 28/50); alveolar/ bronchiolar adenoma or carcinoma (4/50, 28/49, 34/50, 42/50) Circulatory system: hemangioma (0/50, 0/50, 2/50, 3/50); hemangiosarcoma (4/50, 6/50, 17/50, 5/50); hemangioma or hemangiosarcoma (4/50, 6/50, 18/50, 8/50) Harderian gland: adenoma (1/50, 3/50, 3/50, 8/50); adenoma or carcinoma (2/50, 5/50, 3/50, 9/50) Mammary gland: carcinoma (3/50, 4/50, 7/50, 12/50) Liver: hepatocellular carcinoma (4/50, 11/49, 14/50, 19/50); hepatocellular adenoma or carcinoma (20/50, 26/49, 20/50, 30/50) Skin: sarcoma (0/50, 11/50, 11/50, 18/50) Mesentery: sarcoma (0/50, 4/50, 8/50, 3/50) Forestomach: squamous cell papilloma or squamous cell carcinoma (1/50, 0/50, 0/50, 4/50) Zymbal's gland: carcinoma (0/50, 0/50, 0/50, 3/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Chloroprene

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Uncertain findings	Urinary bladder: transitional epithelium carcinoma (0/50, 0/50, 1/50, 0/49); transitional epithelium papilloma (0/50, 0/50, 0/50, 1/49)	Urinary bladder: transitional epithelium carcinoma (0/49, 0/50, 0/50, 2/50) Lung: alveolar/ bronchiolar adenoma (1/49, 0/50, 0/50, 3/50)	None	None
Levels of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutation:			Negative with and without S9 in strains TA98, TA100, TA1535, and TA1537	
Sex-linked recessive lethal mutations				
Drosophila melanogaster:			Negative	
Sister chromatid exchanges				
Mouse bone marrow cells *in vivo*:			Negative in male mice	
Chromosomal aberrations				
Mouse bone marrow cells *in vivo*:			Negative in male mice	
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo* (12 exposures):			Negative in male mice	
Mouse peripheral blood *in vivo* (13-week exposure):			Negative in male and female mice	

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on chloroprene on 11 December 1996 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 11 December 1996, the draft Technical Report on the toxicity and carcinogenesis studies of chloroprene received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.L. Melnick, NIEHS, introduced the toxicology and carcinogenesis studies of chloroprene by discussing the uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and reporting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies were *clear evidence of carcinogenic activity* in male and female F344/N rats and B6C3F_1 mice.

Dr. Ward, a principal reviewer, agreed with the proposed conclusions. He commented that there were many nonneoplastic lesions in the nasal cavity of rats and mice but no nasal neoplasms. He stated that the Discussion section should indicate that many toxic and reparative nasal lesions did not lead to neoplasms, in regard to a current theory/hypothesis that chronic lesions may lead to cancer. Dr. Ward said it was important to know whether the hyperplasias in many organs were focal or diffuse. Dr. Melnick said most of the hyperplasias were focal and this would be emphasized in the text. Because so many tissues were involved, Dr. Ward suggested an additional summary table for comparison to 1,3-butadiene, which might list target organs of toxicity and carcinogenesis. Dr. Melnick agreed.

Dr. Goldsworthy, the second principal reviewer, agreed with the proposed conclusions. He noted the significant changes in survival and body weights that occurred during the study. Dr. Goldsworthy thought the differing decreases in body weights between exposure concentrations might call into question the numbers derived from the dose-response curves. Additionally, he asked for clarification of the impact of *Helicobacter* infection on the interpretation of hepatocellular neoplasms and liver hemangiomas in male mice (see Appendix O).

Dr. Russo, the third principal reviewer, agreed with the proposed conclusions.

Dr. Goldsworthy moved that the Technical Report on chloroprene be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, *clear evidence of carcinogenic activity*. Dr. Russo seconded the motion, which was accepted unanimously with nine votes.